

Enhanced DNA amplification from single cells using the Advalytix Cell Extraction Kit

Based on AmpliGrid technology

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Based on the new advanced AmpliGrid technology, improved DNA amplification is now possible in a low volume reaction format using single cells as a template source. DNA amplification is enhanced thanks to the Advalytix Cell Extraction Kit. The unique enzyme mix efficiently lyses the cells and the DNA gets available for downstream amplification reactions with standard PCR systems as DNA associated proteins are digested. Due to the flexibility of the AmpliGrid technology, cell extraction and PCR can be performed on the identical reaction site of the AmpliGrid AG480F.

Cell isolation

Lymphocytes were isolated from human peripheral blood by PANCOLL (Pan Biotech) density centrifugation and transferred in phosphate buffered saline (PBS, 0.05x).

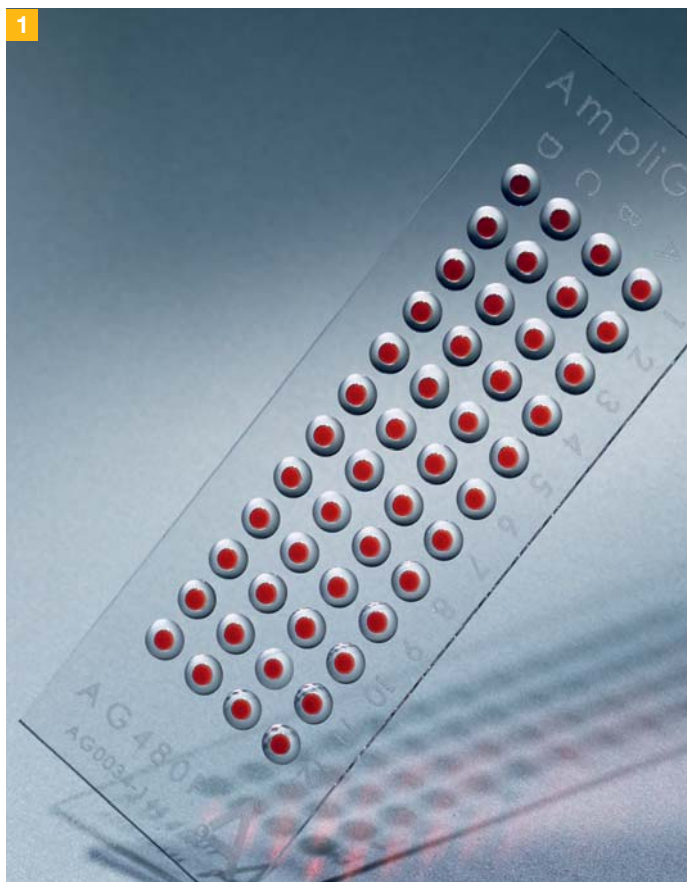


Fig. 1: AmpliGrid AG480F slide

Cell sorting

Single cells were sorted using a MoFlo™ High Performance cell sorter (Dako Cytomation) directly onto each of the 48 AmpliGrid reaction sites (fig. 1). Vital cells were sorted according to their side and forward scatter signals¹.

Cell extraction

The cell extraction working solution was prepared as described in table A.

A Table A: Contents of the cell extraction working solution

	Component Volume (1 slide)	Volume (5 slides)
Lysis Enzyme	1 µL	5 µL
10x Lysis Buffer	6 µL	30 µL
Nuclease free water	53 µL	265 µL
Total volume	60 µL	300 µL

After transferring 0.75 µL of cell extraction working solution to each reaction site on the AmpliGrid slide, every droplet was covered with 5 µL of sealing solution. The loaded AmpliGrid was placed on the AmpliSpeed slide cycler and the cell extraction programme (tab. B) was started. After cell extraction the AmpliGrid was cooled down to room temperature.

B Table B: Cell extraction programme, heating rate 3°C/sec

Temperature	Time
75 °C	5 min
95 °C	2 min
RT	∞

PCR

PCR amplification from the single cells was performed using 6-plex PCR on the Advalytix AmpliSpeed slide cycler (fig. 2). The primer sequences are shown in table C.

A PCR master mix was prepared as described in table D in an approx. 2-fold concentration.

0.75 µL of PCR master mix were transferred to each reaction site on the AmpliGrid slide by pipetting on top of the sealing solution. The water based PCR mix moves through the

sealing solution and merges with the lysis mixture placed on the reaction site. The loaded AmpliGrid was transferred to the AmpliSpeed slide cycler and the PCR programme was started according to table E. After cycling the AmpliGrid was cooled down to room temperature.



Figure 2: AmpliSpeed ASC200D slide cycler (Advalytix)

C Table C: Primer sequences

Primer	Sequence
1	Left 5'-ATACTA ACCATGCGGGTTGC -3' Right: 5'-AGAGGGACAACAAACGTGCT -3'
2	Left: 5'- GTGAGGATTCTGGGCACACT-3' Right: 5'-TGTTTATTCTGGCACTCCAATG -3'
3	Left: 5'-GATAGCAAATGCACCACGG -3' Right: 5'-TTTTCCCGCCTAAAGCATC -3'
4	Left: 5'- AGGCATTGTGGAGATAACGC-3' Right: 5'- AAACATCAAATAGTCCAAGATTCCG-3'
5	Left: 5'-TGGCCCCTGTGTTCAAGT -3' Right: 5'-AGAATTGCTGAAGTGTGTTAGCC -3'
6	Left: 5'-GGTGGATGCTTCTGCCTAAA -3' Right: 5'- TTAGTTATGGGTGCCAAGAT-3'

D Table D: Contents of the master mix

Component	Volume 1 reaction site
2x Qiagen Multiplex PCR Master Mix	0.59 µL
Primermix 2 pmol/µl each	0.1 µL
Q-Solution, 5x	0.06 µL
Total volume	0.75 µL

E Table E: Amplification programme

Temperature	Duration
95 °C	10 min
94 °C	30 sec
63 °C	60 sec 40 cycles
72 °C	60 sec
72 °C	10 min
Ambient	∞

Polyacrylamide gel electrophoresis (PAGE) analysis

After cycling, 4 µL of 1x gel loading dye were loaded on each reaction site on the AmpliGrid slide. The water based loading dye moves through the sealing solution due to its higher density and merges with the reaction mix. 4 µL of the ready-to-load reaction mix were loaded by pipetting through the sealing solution and transferred onto the polyacrylamide gel lanes. After gel electrophoresis PCR products were visualized by silver staining. The results are shown in figure 3.

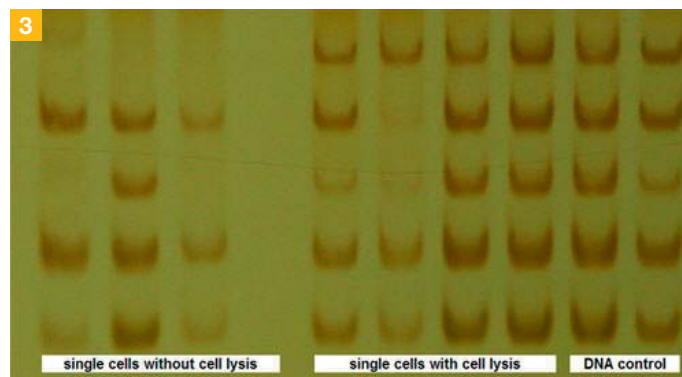


Figure 3: Polyacrylamide gel analysis of PCR products from single cells with or without previous cell extraction

Discussion

Using the Cell Extraction kit in combination with the AmpliGrid technology, a highly sensitive and improved single cell analysis can be performed. While conventional PCR platforms are losing template DNA, the new technology can improve enhanced results in single cell analysis and make the amplification of DNA from single cells much more efficient. DNA bands appear brighter on the gel and the reproducibility from one or few starting copies is increased significantly.

¹ FACS sorting of cells was done by Dr. J.W. Ellwart, Helmholtz Zentrum München GmbH, Ingolstädter Landstraße 1, D-85764 Neuherberg

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